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Bradykinin as a pain mediator: Receptors are localized to sensory neurons, and antagonists have analgesic actions

(spinal cord–dorsal horn/inflammatory pain/hyperalgesia/nociceptors/primary afferents)

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ABSTRACT **Autoradiographic studies localize [^{3}H]bradykinin receptor binding sites to the substantia gelatinosa, dorsal root, and a subset of small cells in both the dorsal root and trigeminal ganglia of the guinea pig. [^{3}H]Bradykinin labeling is also observed over myocardial/coronary visceral afferent fibers. The localization of [^{3}H]bradykinin receptors to nociceptive pathways supports a role for bradykinin in pain mediation. Several bradykinin antagonists block bradykinin-induced acute vascular pain in the rat. The bradykinin antagonists also relieve bradykinin- and urate-induced hyperalgesia in the rat paw. These results indicate that bradykinin is a physiologic mediator of pain and that bradykinin antagonists have analgesic activity in both acute and chronic pain models.**

Inflammatory pain is thought to be initiated by chemical mediators released from damaged tissue that stimulate specific receptor sites on nociceptive sensory neurons. Bradykinin (BK), a nonapeptide (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), has long been thought to be a mediator of inflammatory pain, based on the increased presence of BK in injured tissue (1) and the potent algesic effects of exogenously administered BK (2, 3). While consistent with a role for BK in pain, the evidence is not conclusive. Ideally one should demonstrate BK receptors on sensory fibers and show that inhibition of BK action alleviates pain. In preliminary studies we reported the autoradiographic localization of BK receptors (4, 5) and the antinociceptive effects of BK antagonists (6, 7). We now show that BK receptors are localized to sensory neurons and that a variety of BK receptor antagonists selectively elicit analgesia in several models of tissue damage.

MATERIALS AND METHODS

Receptor Binding. The specific binding of [^{3}H]BK was examined by using the method of Manning *et al.* (8) with minor modifications. Unlabeled BK (1 μM) was used to define nonspecific binding. Data were analyzed as described by McPherson (9).

Autoradiography. For autoradiographic localization of [^{3}H]BK receptor binding, guinea pigs were perfused through the heart with 500 ml of a mixture of equal parts of isotonic phosphate-buffered saline (pH 7.4) and 10% (wt/vol) sucrose. After perfusion, the tissues were removed, embedded in homogenized calf cortex, and frozen on dry ice. Canine tissues were removed without perfusion and immersed in chilled perfusion medium for 20 min, then mounted and frozen as described. Ten-micrometer sections were cut in a cryostat at −12°C, thaw-mounted onto chrome alum/gelatin-coated microscope slides, and stored frozen at −20°C.

When ready for use, the slides were warmed to room temperature, incubated for 120 min at 4°C in medium containing 20 mM 2-{[tris(hydroxymethyl)methyl]amino}ethane sulfonic acid (Tes) (pH 6.8), 1 mM 1,10-phenanthroline, 0.2% bovine serum albumin (protease free), 1 mM dithiothreitol, bacitracin (140 μg/ml), 0.1 mM SQ20,881, 300 mM sucrose, and 0.5 nM [^{3}H]BK (52 Ci/mmol; 1 Ci = 37 GBq). Blanks were incubated in the same medium with 1 μM unlabeled BK or Lys-BK.

After incubation, the tissue sections were washed in a solution of 25 mM Tes (pH 6.8) and 10% (vol/vol) sucrose for two 10-min periods at 4°C, dried rapidly under cold dry air, and apposed to either NTB-3 emulsion-coated coverslips or LKB Ultrafilm for 1 month. After exposure, the coverslip/slide or Ultrafilm was developed, and the tissue was stained with cresyl violet.

Isolated Tissues. Smooth muscle effects of the BK analogs were determined on the isolated rat uterus and isolated guinea pig ileum by standard methods (10). BK analogs were incubated with the preparation for 30 sec before adding an ED_{50} dose of BK. BK analogs that decreased BK were then examined in detail to determine pA_2 values for antagonism (pA_2 is the negative log of the antagonist concentration that causes a 2-fold shift in the agonist dose–response curve) (11). Dose–response curves were determined for analogs that produced contractions at 10 μM.

BK-Induced Vascular Pain. Male Sprague–Dawley rats (200–300 g) (Charles River Breeding Laboratories) were housed five per cage with constant access to food and water with a 12-hr light–dark cycle (7 a.m.–7 p.m.). Rats received an indwelling carotid artery cannula routed subcutaneously to exit behind the head. One to 3 days following surgery, the cannula was connected through tubing to a remote syringe allowing intraarterial injections while the rats were conscious and freely moving. In preliminary experiments, BK at 2 nmol/kg caused stereotypic flexion of the right forelimb and dextrorotation of the head in essentially 100% of the rats tested. Rats that displayed this BK response were injected 5 min later, through the same arterial cannula, with BK and a BK antagonist at 2, 20, or 200 nmol/kg.

BK- and Urate-Induced Hyperalgesia in Rat Paw. Hyperalgesia was measured by a paw-pressure test (12) with a commercially available apparatus (Pressure Analgesia Meter, Stoelting, Chicago). Continuously increasing pressure was applied to the dorsal surface of the paw by a blunt plastic rod until rats withdrew the paw. The indicated pressure at with-

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Abbreviations: BK, bradykinin; Hyp, 4-hydroxyproline; Thi, β-(2-thienyl)alanine; Nal, β-(2-naphthyl)alanine.

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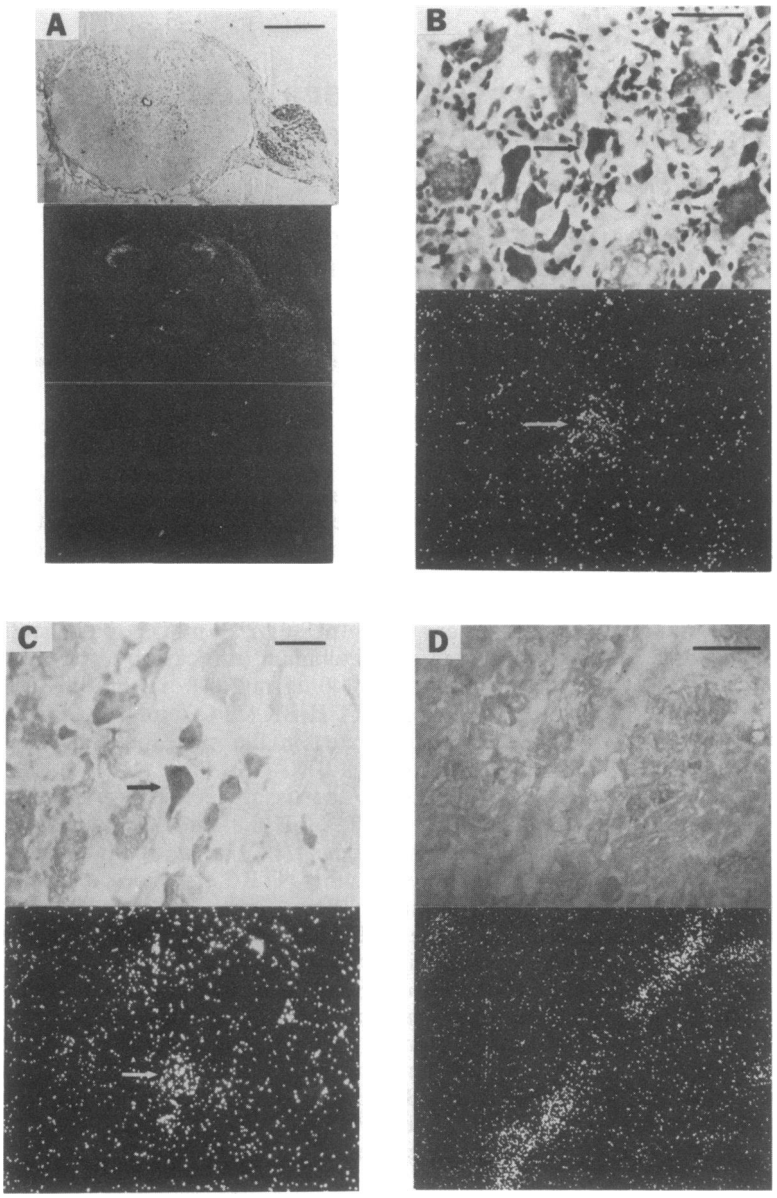

FIG. 1. Autoradiographic distribution of [^{3}H]BK receptor binding sites in guinea pig and canine primary afferent neurons. (*A*) Guinea pig lumbar spinal cord and dorsal root ganglion. (Bar = 1 mm.) (*Top*) Bright-field photomicrograph. (*Middle*) Dark-field photomicrograph from Ultrafilm. Note labeling over substantia gelatinosa, dorsal root, and dorsal root ganglion. (*Bottom*) Dark-field photomicrograph of Ultrafilm incubated with [^{3}H]BK and 0.1 μM unlabeled Lys-BK. (*B*) Guinea pig dorsal root ganglion at high magnification. (Bar = 100 μm.) (*Upper*) Bright-field photomicrograph. (*Lower*) Dark-field photomicrography from NTB3 emulsion-coated coverslip overlying the tissue section in the *Upper* micrograph. Arrows in both photographs indicate a BK-labeled dorsal root ganglion cell. (*C*) Guinea pig trigeminal ganglion at high magnification. (Bar = 50 μm.) (*Upper*) Bright-field photomicrography. (*Lower*) Dark-field photomicrograph from NTB3 emulsion-coated coverslip. Arrows in both photographs indicate a BK-labeled cell. (*D*) Canine stellate ganglion at high magnification displaying both sympathetic ganglion cells and visceral afferent fibers of passage. (Bar = 50 μm.) (*Upper*) Bright-field photomicrograph. (*Lower*) Dark-field photomicrograph from NTB3 emulsion-coated coverslip. Note labeling over fiber tracts and relative absence of labeling over sympathetic ganglion cells.

drawal was recorded as the mechanical pain threshold. For BK-induced hyperalgesia, 20 μl of saline, or saline containing 2 nmol of BK, 2 or 20 nmol of a BK antagonist, or 2 nmol of BK plus 2 or 20 nmol of an antagonist were injected intradermally into the dorsal surface of the right hind paw. Rats were tested at the injection site 5 min after drug administration. For urate-induced hyperalgesia, rats were injected subcutaneously in the dorsal surface of the paw with saline or 10 mg of sodium urate crystals (in 100 μl). BK antagonists (in 20 μl of saline) were injected at the same site, and rats were tested 4 hr later.

Materials. The following BK analogs were used: D-Arg-[Hyp^3,D-Phe^7]BK (NPC-567), D-Arg-[Hyp^2,D-Phe^7]BK (NPC-566), D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK (NPC-349), [D-

Table 1. Effects of BK analogs on [^{3}H]BK binding and isolated tissue contraction

Antagonist		[^{3}H]BK-binding K_i, nM		Antagonist potency, pA_2	
NPC no.	Sequence	N1E-115	GPI	GPI	Rat uterus
349	D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK	3.0 ± 0.8 (2)	19.9 ± 2.1 (3)	6.0	6.9
567	D-Arg-[Hyp^3,D-Phe^7]BK	4.0 ± 0.2 (3)	36.1 ± 0.6 (3)	5.9	6.5
573	[D-Nal^1,Thi^{5,8},D-Phe^7]BK	>5000 (1)	>10,000 (3)	Inactive	5.6
414	Lys-Lys-[Hyp^{2,3},Thi^{5,8},D-Phe^7]BK	25.5 ± 7.6 (4)	84.7 ± 7.9 (3)	5.6	(0.05)
566	D-Arg-[Hyp^2,D-Phe^7]BK	6.5 ± 0.8 (3)	11.5 ± 1.3 (2)	6.3	(2.00)
722	[Leu^{5,8},Gly^6,D-Phe^7]BK	45.5 ± 12.9 (3)	54.2 ± 7.4 (3)	5.9	(0.20)

Specific binding of [^{3}H]BK to receptors on membranes obtained from guinea pig ileum (GPI) and N1E-115 neuroblastoma cells was determined as described (8). The specific binding of [^{3}H]BK was defined as the difference between [^{3}H]BK bound in the absence and presence of 1 μM unlabeled BK. Values shown are the K_i values (mean ± SEM) of the number of independent experiments shown in parentheses. Smooth muscle experiments employed the isolated rat uterus and guinea pig ileum. Analogs that produced a BK-like contraction of the tissue by themselves were assayed as BK agonists; the potencies of these compounds are expressed in parentheses as a percentage of the potency of BK. Antagonist potency is expressed in terms of the pA_2 value for inhibiting BK-induced contraction.

Table 2. Antagonism of BK-induced vascular pain in rats

Antagonist		Inhibition of BK-induced pain, number blocked/number tested		
NPC no.	Sequence	Ant. at 2 nmol/kg	Ant. at 20 nmol/kg	Ant. at 200 nmol/kg
349	D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK	2/4	4/4	4/4
414	Lys-Lys-[Hyp^{2,3},Thi^{5,7},D-Phe^7]BK	6/7	6/7	1/7
566	D-Arg-[Hyp^2,D-Phe^7]BK	3/4	4/4	4/4
567	D-Arg-[Hyp^3,D-Phe^7]BK	1/8	7/7	8/8
573	[D-Nal^1,Thi^{5,8},D-Phe^7]BK	0/5	0/5	0/5
722	[Leu^{5,8},Gly^6,D-Phe^7]BK	5/5	6/6	5/5

Conscious and freely moving rats received BK at 2 nmol/kg in the right carotid artery. Rats that displayed the stereotypic flexion of the right forelimb and dextrorotation of the head were injected 5 min later, through the same route, with BK plus the indicated antagonist (Ant.) at 2, 20, or 200 nmol/kg. The data show the number of rats tested in which the BK effect was blocked relative to the total number of rats tested. Previous studies indicated that the vehicle alone did not alter the frequency of occurrence of the BK effect (unpublished data).

Nal^1,Thi^{5,8},D-Phe^7]BK (NPC-573), Lys-Lys-[Hyp^{2,3},Thi^{5,8}, D-Phe^7]BK (NPC-414), and [Leu^{5,8},Gly^6,D-Phe^7]BK (NPC-722), where Hyp is 4-hydroxyproline, Thi is β-(2-thienyl)-alanine, and Nal is β-(2-naphthyl)alanine. These BK antagonists were synthesized as described (13). Monosodium urate crystals were prepared from uric acid (Sigma) as described (14). BK and the BK antagonists were dissolved in saline, and urate crystals were suspended in saline. [^{3}H]BK (52–76 Ci/mmol) was obtained from New England Nuclear/DuPont. BK, Lys-BK, and des-Arg^9-BK were obtained from Bachem Fine Chemicals (Torrance, CA), and SQ20,881 was purchased from Peninsula Laboratories (Belmont, CA). Low-protease bovine serum albumin and bacitracin (Zn^{2+}-free form) were purchased from Sigma.

RESULTS

Autoradiographic Localization of BK Receptors. High-affinity binding of radiolabeled BK occurs in a variety of tissues in several species (4, 15–17). We have localized specific BK receptor binding to sensory neurons by autoradiography (Fig. 1). [^{3}H]BK-associated silver grains in slices of guinea pig spinal cord and dorsal root ganglion were completely abolished by 0.1 μM BK. As little as 0.1 nM BK, equal to the concentration of [^{3}H]BK employed, reduced grain density by ≈50%, providing evidence for high-affinity binding sites (data not shown). Lys-BK, whose affinity for BK receptors is similar to that of BK (8), at 0.1 μM abolished grain density as effectively as BK itself. By contrast, des-Arg^9-BK (1 μM), inactive at the BK receptors labeled with [^{3}H]BK (8), had no effect on grain density.

[^{3}H]BK receptors were concentrated in a narrow band in the dorsal horn of the spinal cord, the substantia gelatinosa (lamina II), the dorsal root ganglion, and the dorsal root. At high-power magnification, the receptor-associated grains in the dorsal root ganglion were above a subset of small neuronal cells found at greatest density in the dorsal periphery of the ganglion. The absence of silver grains over the spinal ventral root indicates that [^{3}H]BK does not bind to all axons. The grains over sensory fibers peripheral to the dorsal root ganglion indicate a localization of receptors throughout the sensory nerves. The small spur of grains overlying the substantia gelatinosa was consistently observed in various sections and eliminated by unlabeled BK; however, its anatomical correspondence was unclear. These localizations, observed in the lumbar spinal cord (Fig. 1), were essentially the same as BK receptors in cervical, thoracic, and sacral guinea pig spinal cord (data not shown).

We also visualized BK receptors in guinea pig trigeminal ganglion and dog stellate ganglion (Fig. 1). In the trigeminal ganglion, as in the dorsal root ganglion, intense clusters of BK receptor-associated grains were localized to a subset of small neuronal cells. In the stellate ganglion, an intense band of silver grains were over a thin apparently unmyelinated neuronal fiber tract. This ganglion contains cell bodies of sympathetic neurons as well as visceral afferent fibers extending from coronary and myocardial tissues to the spinal cord. We have observed [^{3}H]BK receptor binding in myocardial homogenate and autoradiographic preparations that do not distinguish between myocardial and neuronal binding of [^{3}H]BK.

The Effect of BK Analogues on BK Receptor Binding and Smooth Muscle Responses. Certain synthetic BK derivatives antagonize BK-induced contractions of the estrous rat uterus and guinea pig ileum (7, 13). These derivatives also compete potently for [^{3}H]BK binding to membrane preparations of the guinea pig ileum and a neuroblastoma cell line N1E-115 (7). We have now evaluated a more extensive series of such peptides, comparing their relative receptor binding potencies in a neural cell line and ileum with contractile potencies in ileum and uterus (Table 1). The most potent derivatives of [^{3}H]BK that labeled receptors in cell lines and ileum membranes were NPC-349, NPC-567, and NPC-566 with NPC-414 and NPC-722 displaying somewhat less activity. Relative potencies in blocking BK-induced ileal contractions paralleled potencies at [^{3}H]BK binding sites. Thus, NPC-573, inactive in binding assays, failed to block ileal concentrations. Heterogeneity of BK receptors was apparent in the potent antagonist effects of NPC-573 in the rat uterus despite its inactivity in the ileum. NPC-414, NPC-566, and NPC-722 had potent BK antagonist effects in guinea pig ileum but very weak agonist activity in the rat uterus.

Analgesic Effects of BK Antagonists. We have examined BK antagonist actions in several pain models. BK injection into the right common carotid artery of rats produces stereotyped flexion of the right forelimb and dextrorotation of the head, effects blocked by the analgesics codeine, methadone, phenylbutazone, aspirin, and phenacetin (18). Rats exhibiting the stereotypic response after injections of BK at 2 nmol/kg were injected 5 min later in the same carotid artery with BK at 2 nmol/kg and an antagonist at 1–100 times the BK dose (Table 2). At the lowest dose, NPC-414, NPC-566, and NPC-722 blocked the effects of BK in all or most animals, whereas the other antagonists were less effective. At a 10-fold higher dose, all antagonists except NPC-573 blocked BK effects. Interestingly, at the highest dose tested, NPC-414 became less effective, consistent with the weak agonist effect of this agent in the isolated rat uterus. Intra-arterial injection of BK in humans elicits pain (19), supporting the hypothesis that the stereotypic responses to BK presented here involve pain perception.

Table 3. Antagonism of BK-induced hyperalgesia in rat paw by BK antagonists

Antagonist		Antagonist dose, nmol per paw	Paw-withdrawal threshold, % of control	
NPC no.	Sequence		Saline	BK
—	Saline		99.9 ± 2.8 (44)	58.4 ± 2.6* (43)
567	D-Arg-[Hyp^3,D-Phe^7]BK	2	107.3 ± 9.8 (8)	105.0 ± 4.6† (9)
		20	85.0 ± 7.8 (9)	83.0 ± 10.3‡ (9)
349	D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK	2	86.1 ± 4.2 (9)	93.1 ± 8.0† (9)
		20	69.4 ± 3.5* (9)	99.8 ± 12.5† (9)
573	[D-Nal^1,Thi^{5,8},D-Phe^7]BK	20	84.0 ± 3.8 (9)	89.5 ± 10.2† (9)

Rats were injected intradermally into the dorsal surface of the right hind paw with 20 μl of saline, saline containing 2 nmol of BK, the antagonist, or 2 nmol of BK plus the antagonist. The amount of pressure applied to the paw at the injection site that caused paw withdrawal was determined 5 min after drug treatment (12). An independent experiment was done for each dose of each antagonist. Since the pressure threshold in rats treated with saline or BK alone did not differ among the experiments, results from these treatment groups were combined for presentation. Statistical analyses were conducted before the results were pooled. In saline-treated rats, paw withdrawal occurred at 116 ± 6 g of applied pressure. The values shown are the mean ± SEM of the number of rats shown in parentheses.

*$P < 0.05$ compared to saline-treated rats by using Tukey's HSD test following a significant interaction term in a two-way analysis of variance.

†$P < 0.05$ compared to BK-treated rats following a significant interaction term.

‡Significant interaction term in a two-way analysis of variance indicates significant ($P < 0.05$) antagonism.

We examined the effects of antagonists on BK-induced hyperalgesia in the rat paw (Table 3). We compared the nociceptive effects of the pressure stimulus in the presence or absence of BK. BK (2 nmol) or saline was injected intradermally into the dorsal surface of the right hind paw alone or with an antagonist. Five minutes later, pressure was applied to the paw at the injection site, and the paw-withdrawal threshold was determined. At doses 1–10 times higher than that of administered BK, NPC-567 and NPC-349 antagonized the hyperalgesic effect of BK, an effect also observed with 20 nmol of NPC-573. At 20 nmol, NPC-349 lowered the paw-withdrawal threshold in the absence of BK, suggesting partial agonist activity.

To evaluate the role of endogenous BK in pain elicited by physiologic stimuli, we elicited hyperalgesia by injecting urate crystals into the hind paw of rats (Table 4). NPC-349 was the most efficacious antagonist, completely blocking urate effects at 2 nmol. All the other antagonists, except NPC-566, inhibited urate hyperalgesia at 2 nmol. At the 20-nmol dose, all the derivatives exhibited increased inhibition of urate hyperalgesia.

Table 4. Antagonism of urate-induced hyperalgesia in rat paw by BK antagonists

Antagonist		Antagonist dose, nmol per paw	Paw-withdrawal threshold, % of control	
NPC no.	Sequence		Saline	BK
567	D-Arg-[Hyp^3,D-Phe^7]BK	0	100.0 ± 5.6	54.7 ± 4.5*
		2	92.2 ± 7.7	71.4 ± 7.5*
		20	88.2 ± 6.5	86.2 ± 5.1†
349	D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK	0	100.0 ± 5.6	67.4 ± 3.9*
		2	83.9 ± 4.6	98.2 ± 4.2†
		20	89.9 ± 6.0	99.5 ± 10.5†
573	[D-Nal^1,Thi^{5,8},D-Phe^7]BK	0	100.0 ± 5.1	35.8 ± 6.9*
		2	103.2 ± 10.3	54.7 ± 6.4*
		20	103.9 ± 6.4	76.5 ± 2.8†
414	Lys-Lys-[Hyp^{2,3},Thi^{5,8},D-Phe^7]BK	0	100.0 ± 4.6	47.2 ± 6.2*
		2	74.0 ± 8.1	74.5 ± 14.1‡
		20	77.8 ± 6.2	79.9 ± 8.7‡
722	[Leu^{5,8},Gly^6,D-Phe^7]BK	0	100.0 ± 2.9	64.7 ± 2.5*
		2	84.4 ± 8.5	79.8 ± 4.7‡
		20	90.7 ± 5.6	83.0 ± 5.7‡
566	D-Arg-[Hyp^2,D-Phe^7]BK	0	100.0 ± 3.8	45.5 ± 3.7§
		2	87.8 ± 6.1	44.3 ± 6.4§
		20	93.8 ± 8.6	57.4 ± 4.1§

Rats were injected subcutaneously into the dorsal surface of the right hind paw with saline or sodium urate crystals (10 mg in 100 μl of saline) plus saline or plus the antagonist. The amount of pressure applied to the paw at the injection site that caused paw withdrawal was determined 4 hr after drug treatment (12). An independent experiment was done for each antagonist. In saline-treated rats, paw withdrawal occurred at 112 ± 6 g of applied pressure. The values shown are the means ± SEM of results from 6 to 10 rats.

*$P < 0.05$ compared to saline-treated rats by using Tukey's HSD test following a significant interaction in a two-way analysis of variance.

†$P < 0.05$ compared to BK-treated rats following a significant interaction term.

‡Significant interaction term in two-way analysis of variance indicates significant ($P < 0.05$) antagonism.

§$P < 0.05$ compared to saline-treated rats as indicated by a significant overall BK effect with no interaction.

Table 5. Inhibition of urate-induced hyperalgesia by NPC-567 administered after urate

Treatment	Paw-withdrawal threshold, % of control
Saline–saline	100.0 ± 7.5
Urate–saline	57.1 ± 6.9*
Saline–NPC-567	106.9 ± 5.8
Urate–NPC-567	95.3 ± 7.5†

Rats were injected s.c. into the dorsal surface of the right hind paw with saline or 10 mg of sodium urate crystals followed 2 hr later by the s.c. administration of saline or 20 nmol of NPC-567. The amount of pressure applied to the paw at the injection site that caused paw withdrawal was determined 2 hr after NPC-567 administration (12). Paw withdrawal in saline-treated rats occurred at 107 ± 8 g of applied pressure. The values shown are the means ± SEM of results from eight or nine rats.

*$P < 0.05$ compared to saline-treated rats by using Tukey's HSD test following a significant interaction term in a two-way analysis of variance.

†$P < 0.05$ compared to rats treated with urate alone.

In clinical gout, urate crystals are present for a substantial period of time before analgesic therapy. Accordingly, we administered NPC-567 2 hr after injection of sodium urate crystals (Table 5). At the time of maximal hyperalgesia, NPC-567 (20 nmol) completely reversed the hyperalgesic effects of urate.

DISCUSSION

The present study strongly supports the role of BK as a physiological pain mediator. BK receptors are localized to areas enriched in nociceptive sensory neurons. Nociceptive neural transmission involves small-diameter unmyelinated or thinly myelinated fibers with small cell bodies in the dorsal root ganglion that terminate in the superficial layers of the dorsal spinal cord. By contrast, touch and pressure sensation is conveyed by thick myelinated fibers with large cell bodies in the dorsal root ganglion and terminals in relatively deep layers of the dorsal spinal cord. BK receptors are selectively localized to sites involved in nociception: superficial layers of the spinal cord, thin unmyelinated fibers, and small neuronal cells in sensory ganglia. BK has been implicated in cutaneous and visceral pain, consistent with receptor localizations observed here. While nociceptive sensory fibers terminate in trigeminal ganglion cells, such fibers from the heart fully transverse the stellate ganglion, whose major cells are sympathetic (20). This pattern fits with BK receptor localization to small cells in trigeminal ganglia and to fibers but not cells in the stellate ganglion. Peripheral terminals of primary afferents presumably contain the BK receptors that respond directly to tissue injury and inflammation. Centrally directed axonal transport can account for BK receptors visualized in the spinal cord.

Blockade by BK derivatives of BK-induced acute vascular pain and hyperalagesia indicates that these peptides are selective BK antagonists *in vivo*. While most of the analogs tested showed potent activity in both vascular and hyperalgesia models, NPC-573 failed to block BK-induced vascular pain or ileal contractions but antagonized BK-induced rat-paw hyperalgesia and BK contraction of the rat uterus. Thus, the BK receptor involved in vascular pain may be different from the receptor involved in cutaneous hyperalgesia.

Because the antagonists block urate hyperalgesia, endogenous BK appears involved in hyperalgesia. The urate crystals provide a nidus for kallikrein activation in the tissue with subsequent production of BK and nociceptor sensitization (2). BK antagonists block the urate hyperalgesia even when administered after the development of an altered pain sensitivity, suggesting that BK must be continuously produced to maintain the hyperalgesic state. BK antagonists also block acetic acid-induced writhing, another model of pathologic pain that predicts clinical analgesic activity (6). With the release of BK following tissue injury (1) and with the extremely potent algesic effects of BK (2, 3, 21), the present findings establish a convincing case for BK as a physiologic pain mediator.

The receptor localizations reported here could account for pain transmission in specific pathologic conditions such as angina and renal colic. Coronary artery constriction causes release and accumulation of BK in the coronary vasculature and myocardium (22). Epicardial administration of BK stimulates cardiovascular visceral afferent nociceptors (23) that travel to the spinal cord through the stellate ganglion. Clinically intractable angina can be relieved by lesioning the stellate ganglion (20). Renal colic may involve BK receptors highly concentrated in the subendothelial layer of the ureter (5). The ureteral subendothelium contains a dense plexus of presumably nociceptive unmyelinated fibers likely to represent the localization of BK receptors.

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